PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

CANNING, Lewis R. et al
Amersham plc
Amersham Place
Little Chalfont HP7 9NA
GRANDE BRETAGNE

DUE DATE:	—
FORMALITIES:	FISL ✓
PAT. OFF:	LRC /F
ON DB:	—
CASE NO:	PZ0277-PCT

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)* 26.01.2005

Applicant's or agent's file reference
PZ0277-PCT

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 03/04351	08.10.2003	08.10.2002

Applicant
AMERSHAM PLC et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Morancho Alcaine, N

Tel. +49 89 2399-7462

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PZ0277-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04351	International filing date *(day/month/year)* 08.10.2003	Priority date *(day/month/year)* 08.10.2002
International Patent Classification (IPC) or both national classification and IPC A61K31/515		
Applicant AMERSHAM PLC et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.03.2004	26.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Loher, F Telephone No. +49 89 2399-7839

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04351

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-55 as originally filed

Claims, Numbers

1-32 filed with telefax on 03.11.2004

Drawings, Sheets

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 30-32 (IA)

   because:

   ☒ the said international application, or the said claims Nos. 30-32 (IA) relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-8, 10-14, 17, 18, 23-32
	No:	Claims	1, 2, 9, 15, 16, 19-22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-32
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the report

Art 34(2)(b) The amendments filed with telefax dated 03.11.2004 do not introduce subject-matter which extends beyond the content of the application as filed. Therefore, the requirements of Article 34(2)(b) PCT are met. Present claims 1-32 have been examined.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 30-32 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: Biological Chemistry (08-2001), 382(8), 1277-1285
D2: Bioorganic And Medicinal Chemistry Letters (23-04-2001), 11(8), 969-972
D3: Journal Of Nuclear Medicine (1981), 22(6), P12-P13
D4: EP-A-0293971
D5: US-A-3952091
D6: US-A-4124760
D7: WO-A-9960018

If not mentioned otherwise, the relevant passages are those mentioned in the International Search Report.

Art 33(2) The present application does not meet the requirements of Article 33(2) PCT, since the subject-matter of claims 1, 2, 9, 15, 16 and 19-22 is not new.

D1 discloses MMP inhibiting barbituric acid derivatives. In particular compound 16 (but also compounds 15 and 17-26) is nearly identical with precursor compounds disclosed in the present application (see e.g. present compound 6 or 11). Present claim 19 merely defines a barbituric acid MMP inhibitor being conjugated to a ligand which is capable of forming a metal complex. Consequently, the metal complex is not a distinguishing feature. The desideratum definition of the ligand "forming a metal complex ... which is resistant to transchelation" is not suitable to render present claims 19-22 novel over D1.
However, D1 does not mention the feature "sterile form" of present claim 26 which corresponds to original claim 24.
In summary, the subject-matter of claims 19-22 is not new in the light of D1.

D2 discloses barbituric acid derivatives being substituted at the 5 position by a ligand which is capable of a reaction with radioactive non-metal. For the same reasons as mentioned above, the subject-matter of present claims 19-22 is not new in the light of D2.

D3 discloses barbituric acid derivatives having an imaging moiety (radioactive non metal) linked to position 5 of the barbituric acid. As selenium and tellurium isotopes are gamma radiation emitting non-metals, the IPEA agrees that the disclosure of D3 does not fall within the scope of present claim 1 or 26. Therefore, the disclosure of D3 is not novelty-destroying for the claims of the present application.

D4 discloses Tritium-labelled phenobarbital which is capable of a reaction with radioactive non-metal. The applicant alleges that "tritium is not an imaging moiety suitable for detection following administration *in vivo*". Examiner stress that the European Pharmacopoeia (2002), lists tritium labelled water for intravenous admistration (determination of body water which is *in vivo* detection). In consequence the objection made by the examiner in the Written Opinion is upheld. Therefore, the subject-matter of claims 1, 2, 9, 15 and 16 is

not considered to be new in the light of D4.

Art 33(3) The present application does not meet the requirements of Article 33(3) PCT, since the subject-matter of claims 1-32 does not seem to involve an inventive step.

D7 discloses the concept of using an imaging moiety containing ligands of the present invention bound to different biologically active compounds in the manufacture of radiopharmaceuticals. The thus obtained radiopharmaceuticals are *inter alia* useful in the diagnosis of atherosclerosis. Consequently, D7 discloses the concept of targeting imaging moieties. Therefore it is still considered to be appropriate to start the problem solution approach from D7.

The problem to be solved by the present invention may therefore be regarded as how to provide improved radiopharmaceuticals for the use in the diagnosis of atherosclerosis.

The present application suggests to solve the problem posed by providing barbituric acid being substituted at the 5 position by a ligand that comprises a imaging moiety or by a ligand which is capable of a reaction with radioactive non-metal or which is capable of forming a complex with a radioactive or paramagnetic metal.

D1 and D2 disclose the MMP binding properties of barbituric acids. The overexpression of MMPs in atherosclerotic plaques was known at the priority date of the present application (see description page 2, lines 25-30)

Taking into account the teaching of the cited prior art the following reasoning applies:

With respect to the subject-matter of claims 1, 2, 9, 15, 16 and 19-22 the applicant's attention is drawn to the fact that even if novelty could be established over the above-cited prior art it is at present not clear wherein an inventive step may reside.

With respect to the subject-matter of the remaining claims 3-8, 10-14, 17, 18 and 23-32 the applicant's attention is drawn to the fact that there seems to be no basis for inventive step within the present application as filed since no evidence can be found that the features which are novel result in a solution of the posed problem which could not have been foreseen by the skilled person. Being aware of the fact that MMPs are a potential target for

radiopharmaceuticals useful in the diagnosis of atherosclerosis the skilled man performed an arbitrary choice out of one list containing all known MMP inhibitors to select barbituric acid derivatives. The structural features of the ligands and radioactive compounds has been anticipated by D7.
It is therefore noted, that the solution proposed in claims of the present application is not considered to be inventive in the sense of Article 33(3) PCT.

But even when starting from D3 as the applicant suggests claims 1-18 and 23-32 do not seem to involve an inventive step. D3 discloses the use of radioactive gamma-emitting non-mteals being conjugated to barbituric acids as *in vivo* diagnostics. The subject-matter differs from that of the present application in that D3 does not use imaging moieties which are chosen from items (i)-(vii) as defined by present claim 1.
The problem to be solved could - again - be regarded as how to provide improved radiopharmaceuticals for the use in the diagnosis of atherosclerosis. The present aplication suggests to solve the problem by using imaging moieties as defined by present claim 1.
This solution is not considered to be inventive as no evidence can be found that the features which are novel result in a solution of the posed problem which could not have been foreseen by the skilled person. the use of imging moieties as defined by present claim 1 are well-known in the field of radiopharmaceuticals. Since there is no surprising effect resulting from that choice the solution as suggested by the present application cannot be considered as being inventive.

Art 33(4) For the assessment of the present claims 30-32 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

The subject-matter of claims 1-29 is considered to be industrially applicable in the sense of Art 33(4) PCT.

CLAIMS (revised).

1. An imaging agent which comprises a synthetic barbituric acid matrix metalloproteinase inhibitor labelled at the 5-position of the barbituric acid with an imaging moiety, wherein the imaging moiety can be detected following administration of said labelled synthetic barbituric acid matrix metalloproteinase inhibitor to the mammalian body *in vivo*, and said imaging moiety is chosen from:

   (i) a radioactive metal ion;
   (ii) a paramagnetic metal ion;
   (iii) a gamma-emitting radioactive halogen;
   (iv) a positron-emitting radioactive non-metal;
   (v) a hyperpolarised NMR-active nucleus;
   (vi) a reporter suitable for *in vivo* optical imaging;
   (vii) a β-emitter suitable for intravascular detection.

2. The imaging agent of Claim 1, where the synthetic barbituric acid matrix metalloproteinase inhibitor ligand conjugate is of Formula I:

$$[\{\text{inhibitor}\}\text{-}(A)_n]_m\text{-}[\text{imaging moiety}] \quad (I)$$

where:

{inhibitor} is the synthetic barbituric acid matrix metalloproteinase inhibitor;

$-(A)_n-$ is a linker group wherein each A is independently $-CR_2-$, $-CR{=}CR-$, $-C{\equiv}C-$, $-CR_2CO_2-$, $-CO_2CR_2-$, $-NRCO-$, $-CONR-$, $-NR(C{=}O)NR-$, $-NR(C{=}S)NR-$, $-SO_2NR-$, $-NRSO_2-$, $-CR_2OCR_2-$, $-CR_2SCR_2-$, $-CR_2NRCR_2-$, a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, a C_{5-12} arylene group, or a C_{3-12} heteroarylene group, an amino acid or a monodisperse polyethyleneglycol (PEG) building block;

R is independently chosen from H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

n is an integer of value 0 to 10; and

m is 1, 2 or 3.

3. The imaging agent of Claims 1 or 2, where the synthetic barbituric acid matrix metalloproteinase inhibitor is conjugated to a ligand, and said ligand forms a metal complex with the radioactive metal ion or paramagnetic metal ion.

4. The imaging agent of Claim 3, where the ligand is a chelating agent.

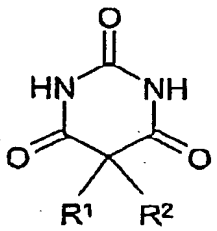
5. The imaging agent of Claims 3 or 4, where the radioactive metal ion is a gamma emitter or a positron emitter.

6. The imaging agent of Claim 5, where the radioactive metal ion is ^{99m}Tc, ^{111}In, ^{64}Cu, ^{67}Cu, ^{67}Ga or ^{68}Ga.

7. The imaging agent of Claims 1 or 2, where the gamma-emitting radioactive halogen imaging moiety is ^{123}I.

8. The imaging agent of Claims 1 or 2, where the positron-emitting radioactive non-metal is chosen from ^{18}F, ^{11}C or ^{13}N.

9. The imaging agent of Claims 1 to 8, where the synthetic barbituric acid matrix metalloproteinase inhibitor is of Formula IV:



(IV)

where:

R^1 is R" or a Z group;

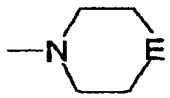
R^2 is R", Y or $-NR^4R^5$, where R^4 is H or an R" group, R^5 is H, C_{2-14} acyl, C_{2-10} aminoalkyl or (N-C_{2-14} acyl)C_{2-10} aminoalkyl

or an R" group, or R^4 and R^5 together with the N atom to which they are attached form an optionally (N-C_{2-14})acylated C_{2-8} cycloaminoalkylene ring;

R" is independently C_{1-14} alkyl, C_{3-8} cycloalkyl, C_{2-14} alkenyl, C_{1-14} fluoroalkyl, C_{1-14} perfluoroalkyl, C_{6-14} aryl, C_{2-14} heteroaryl or C_{7-16} alkylaryl;

Z is a group of formula $-A^1O[A^2O]_pR^3$ where p is 0 or 1, and A^1 and A^2 are independently C_{1-10} alkylene, C_{3-8} cycloalkylene, C_{1-10} perfluoroalkylene, C_{6-10} arylene or C_{2-10} heteroarylene, and R^3 is an R group where R is independently chosen from H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

Y is a group of formula:

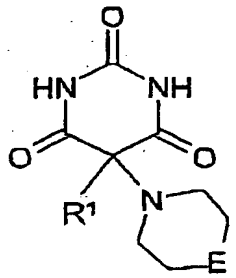


where E is CR_2, O, S or NR^6; and R^6 is C_{2-14} acyl, or an R" or Z group.

10. The imaging agent of claim 9, where R^2 is Y or $-NR^4R^5$.

11. The imaging agent of claims 9 or 10, where the imaging moiety is attached to the R^2 substituent.

12. The imaging agent of claims 9 to 11, of Formula V:



(V)

where E is CHR or NR^6 and R^1 is C_{6-14} *n*-alkyl, or C_{6-14} aryl.

13. The imaging agent of claim 12, where E is NR^6 and R^6 is C_{2-14} acyl; $-(CH_2)_dOH$, where d is 2, 3, 4 or 5; or $-C_6H_4X$, where X is H, C_{1-4} alkyl, Hal, OR, NR_2, NO_2 or $SO_2NR^7R^8$, where R^7 and R^8 are independently R groups, and R is as defined in Claim 9.

14. The imaging agent of claims 12 or 13, where R^1 is *n*-octyl, *n*-decyl, biphenyl, C_6H_5X or $-C_6H_4-O-C_6H_4X$ where X is as defined in Claim 13.

15. A pharmaceutical composition which comprises the imaging agent of claims 1 to 14 together with a biocompatible carrier, in a form suitable for mammalian administration.

16. A radiopharmaceutical composition which comprises the imaging agent of claims 1 to 14 wherein the imaging moiety is radioactive, together with a biocompatible carrier, in a form suitable for mammalian administration.

17. The radiopharmaceutical composition of claim 16, where the imaging moiety comprises a radioactive metal ion.

18. The radiopharmaceutical composition of claim 16, where the imaging moiety comprises a positron-emitting radioactive non-metal or a gamma-emitting radioactive halogen.

19. A conjugate of a synthetic barbituric acid matrix metalloproteinase inhibitor with a ligand, wherein the barbituric acid comprises a 5-position substituent, and said 5-position substituent comprises a ligand capable of forming a metal complex with a radioactive or paramagnetic metal ion which is resistant to transchelation.

20. The conjugate of Claim 19, of Formula Ib:

$$[\{\text{inhibitor}\}\text{-}(A)_n]_m\text{-}[\text{ligand}] \quad \text{(Ib)},$$

where {inhibitor}, A, n and m are as defined in Claim 2.

21. The conjugate of Claims 19 or 20, wherein the synthetic barbituric acid matrix metalloproteinase inhibitor is of Formula IV or Formula V of Claims 9 to 14.

22. The conjugate of Claims 19 to 21, wherein the ligand is a chelating agent.

23. The conjugate of Claim 22, wherein the chelating agent has a diaminedioxime, N_2S_2, or N_3S donor set.

24. A kit for the preparation of the radiopharmaceutical composition of Claim 17, which comprises the conjugate of Claims 19 to 23.

25. The kit of Claim 26, where the radioactive metal ion is ^{99m}Tc, and the kit further comprises a biocompatible reductant.

26. A kit for the preparation of the radiopharmaceutical composition of Claim 18, which comprises a precursor in sterile form which is a non-radioactive derivative of the barbituric acid matrix metalloproteinase inhibitor of claims 1 to 14, wherein said non-radioactive derivative is capable of reaction with a source of the positron-emitting radioactive non-metal or gamma-emitting radioactive halogen to give the desired radiopharmaceutical.

27. The kit of Claim 26, where the source of the positron-emitting radioactive non-metal or gamma-emitting radioactive halogen is chosen from:

(i) halide ion;

(ii) F^+ or I^+; or

(iii) an alkylating agent chosen from an alkyl or fluoroalkyl halide, tosylate, triflate or mesylate;

(iv) $HS(CH_2)_3{}^{18}F$.

28. The kit of claims 26 or 27, wherein the non-radioactive derivative is chosen from:

(i) an organometallic derivative such as a trialkylstannane or a trialkylsilane;

(ii) a derivative containing an alkyl or aryl iodide or bromide, alkyl tosylate or alkyl mesylate for nucleophilic substitution;

(iii) a derivative containing an aromatic ring activated towards nucleophilic or electrophilic substitution;

(iv) a derivative containing a functional group which undergoes facile alkylation;

(v) a derivative which undergoes alkylation with an alkyl thiol to give a thioether.

29. The kit of claims 26 to 28, where the precursor is bound to a solid phase.

30. Use of the imaging agent of Claims 1 to 14 for the diagnostic imaging of atherosclerosis.

31. Use of the imaging agent of Claims 1 to 14 for the diagnostic imaging of unstable plaques.

32. Use of the imaging agent of Claims 1 to 14 for the intravascular detection of atherosclerosis.